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Computer graphics modelling of human renin

Specificity, catalytic activity and intron-exon junctions

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A model has been constructed using computer graphics for human renin based on the sequence derived from that of the gene and the 3-dimensional structure defined at high resolution for other homologous aspartic proteinases. Human renin can adopt a 3-dimensional structure close to that of other aspartic proteinases, in which amino acids corresponding to intron-exon junctions in the gene are at surface regions in the 3-dimensional structure. As expected, the essential catalytic residues are retained and the nearby residue 304 is alanine as in the mouse sequence, supporting the idea that Asp 304 of other aspartic proteinases may contribute to the low pH of their optimal activity. There are interesting differences at subsite S_3' which may contribute to the specificity of human renin. Certain residues at the surface of the enzyme adjacent to the active site cleft are unique to renins and may play a role in recognition and binding of angiotensinogen.

Human renin *Computer graphics model* *Intron-exon junction* *Enzyme specificity*

1. INTRODUCTION

The aspartic proteinase, renin, catalyses the first, and rate-limiting step, in the conversion of angiotensinogen to angiotensin II. As the latter is important in the regulation of blood pressure, the design of renin inhibitors is of great pharmacological interest. Some progress has been made by modification of angiotensinogen sequences [1] especially by the introduction of modified amino acids such as statine residues [2] and reduced peptide bonds [3]. However, a more rational approach would be based on a knowledge of the enzyme–substrate transition state complex.

The first steps in this direction were the determination of the amino acid [4] and cDNA [5] sequences of mouse submaxillary renin. The high homology of these sequences with those of other aspartic proteinases indicated that the enzymes might have similar 3-dimensional structures, and enabled models to be constructed using computer graphics [6,7]. The catalytic residues (Asp 32 and Asp 215, pepsin numbering) lie in the centre of a deep and extended cleft, which can accommodate 7 or 8 amino acids [6]. However, residue 304, which in other aspartic proteinases is invariant as aspartate, was uniquely alanine in mouse submaxillary renin; this may contribute to the higher pH optimum for catalytic activity. In most aspartic proteinases, subsite S_3', residue 189 is usually phenylalanine or tyrosine, but in mouse submaxillary renin it is a much smaller serine, complementing the tyrosine in angiotensinogen. The predicted 3-dimensional structure of mouse submaxillary

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renin has been used as a basis for modelling renin substrate interactions [8]. Mouse kidney renin, which is a separate gene product [9,10], resembles mouse submaxillary renin in specificity, catalytic activity, and sequence (C. Burt and W.J. Brammar, unpublished) [11].

A detailed understanding of the specificity of the human enzyme depends on knowledge of the amino acid sequence and this has now become available from the genomic DNA sequence [12] and from two cDNA sequences [13,14]. Here, we describe a model constructed using interactive computer graphics for human renin based on the sequence derived from that of the gene [12]. We show that human renin can adopt a 3-dimensional structure close to that of the other aspartic proteinases in which amino acids corresponding to intron-exon junctions in the gene are at surface regions in the 3-dimensional structure. We discuss the specificity subsites and various residues on the surface of the enzyme adjacent to the active site cleft which might play a role in recognition and binding of angiotensinogen. We believe that the model provides a useful guide to the rational design of inhibitors of human renin which may be of value in the treatment of hypertension.

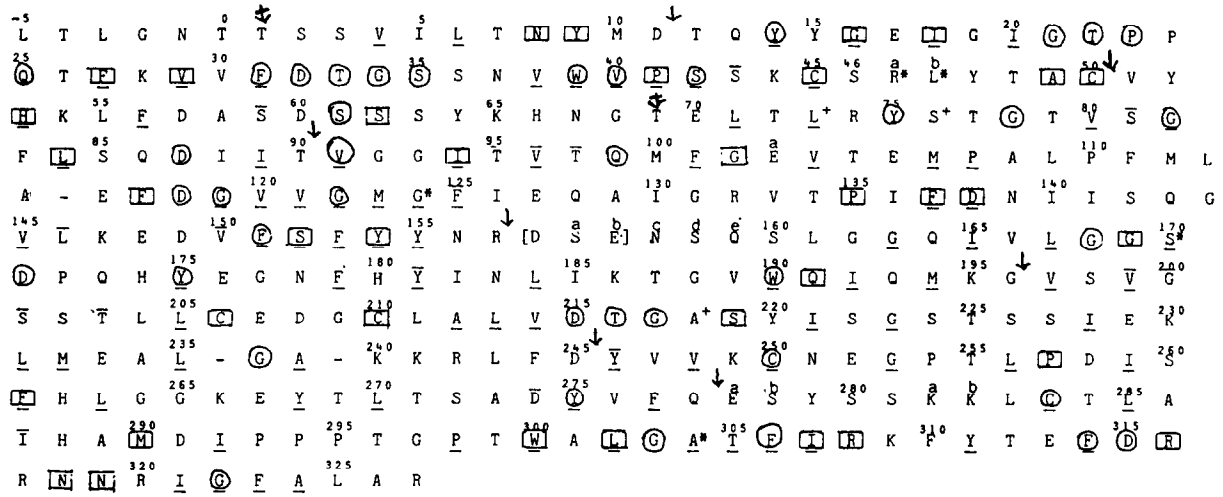
2. MATERIALS AND METHODS

The sequence of human renin determined in [12] was aligned with that of mouse submaxillary renin and other aspartic proteinases in a 3-stage interactive process. First, the sequences of mouse submaxillary and human renins were aligned together as in [14]. Second, the renin sequences were aligned with those of other aspartic proteinases including porcine pepsin [15], human pepsin [16], chicken pepsin [17], calf chymosin [18], endothiapepsin (V. Pedersen and L. Pearl, unpublished) and penicillopepsin [19]. Table 1 indicates residues which are conserved (i) in all aspartic proteinases, (ii) only in mammalian enzymes and (iii) in all aspartic proteinases except the renins. The sequence of human renin has been numbered using the sequence of porcine pepsin with insertions indicated by alphabetical postscripts.

The third stage of the alignment depended on reference to the known structures of other aspartic proteinases defined by X-ray analysis. These include a medium-resolution unrefined model of porcine pepsin [20] and high-resolution structures of rhizopuspepsin [21], penicillopepsin [22] and endothiapepsin [23]. As only the α-carbon coordinates were available for porcine pepsin, and no sequence has been determined for rhizopuspepsin, we did not use these structures extensively. In the third stage of the alignment we attempted to retain the hydrophobic residues of the core of these enzymes as non-polar and to limit insertions and deletions to surface regions and loops. The alignment is indicated in table 1 by the pepsin numbering system.

The 3-dimensional structure of human renin was then modelled using FRODO [24] modified by Dr I.J. Tickle and Dr T.A. Jones for an Evans and Sutherland Picture System 2. We used the 3-dimensional atomic coordinate set of endothiapepsin [23] refined to an agreement (R) value of 0.16 at 2.1 Å resolution as a basis for the model construction. Firstly, the alignment of sequences was used to identify the residues which are identical in endothiapepsin and human renin. Secondly, other residues of the hydrophobic core were replaced in the model by those of human renin. The positions of their side chains were adjusted interactively so that they occupied a position as close as possible to that of the side chains of endothiapepsin while adopting acceptable torsion angles and interatomic distances with non-bonded atoms. Thirdly, insertions and deletions were made using FRODO. In most cases this involved extending or shortening hairpin loops by one or two amino acid residues: the main chain parameters were guided by analyses of other β-bend conformations (B.L. Sibanda and J.M. Thornton, unpublished) and by comparison with other aspartic proteinases as suggested in [25]. Finally, all surface side chains were added in positions which approximated those of endothiapepsin. Ion pair interactions and hydrogen bonds were optimised, torsion angles given preferred values, and disallowed interatomic contacts avoided. We experimented with the use of energy-minimization programs to optimise the interatomic interactions using the potential functions developed in [26], in which hydrogen atoms were explicitly considered for all residues in the active site region. However, these led to unacceptable movements of the water molecules in the active site.

Table 1
Human renin



- deletions compared to porcine pepsin
Conserved in all mammalian aspartic proteinases.
Conserved in all mammalian aspartic proteinases and in endothiapepsin.
X Hydrophobic core.
X* Unique to all renins; conserved in all other aspartic proteinases.
X̄ Identical in human renin and endothiapepsin, but different in pepsin.
X+ Varied only in human renin.

‡, presumptive *O*-glycosylation sites. ↓, positions of intron-exon junctions. The numbering system is derived from that of porcine pepsin and indicates the alignment of the sequences. The sequence within parentheses is not found in the gene sequence of [12], but is in both cDNA sequences [13,14]

The model of human renin was used as the basis for a substrate docking experiment. The residues P_4–P_1 were placed by analogy with the inhibitors bound to rhizopuspepsin [27] and penicillopepsin [28]. This brought the scissile bond into close juxtaposition with a water molecule bound to the two active site aspartates [29]. The water was assumed to participate in a tetrahedral intermediate at the peptide carbonyl. The substrate residues (P_1'–P_3') were then fitted into the specificity pockets in the remaining half of the cleft. The subsite, S_1', was assumed to comprise residues in the region of 213 and 301 while subsite S_3' must be close to 189 [30,31].

3. RESULTS AND DISCUSSION

Fig.1 shows orthogonal stereo views of the model of human renin.

The alignment of the human renin sequence with that of the other aspartic proteinases was achieved in most places without ambiguity owing to the very few insertions and deletions that have occurred between the enzymes of fungal and mammalian origins. When compared to the alignment used in modelling the mouse submaxillary renin [6] there is only one difference. This occurs in residues 157–188 (pepsin numbering) where the existence of two similar renin sequences (mouse submaxillary and human) implied that Gly 168, Asp 171 and Tyr 175 should be invariant in these as well as other aspartic proteinases. This necessitated two insertions at the β-bend between strands 147–156 and 162–171. However, in the region of residues 103–117 where the homology between different aspartic proteinases is low, there is still a real ambiguity in alignment. We have chosen to make the alignment:

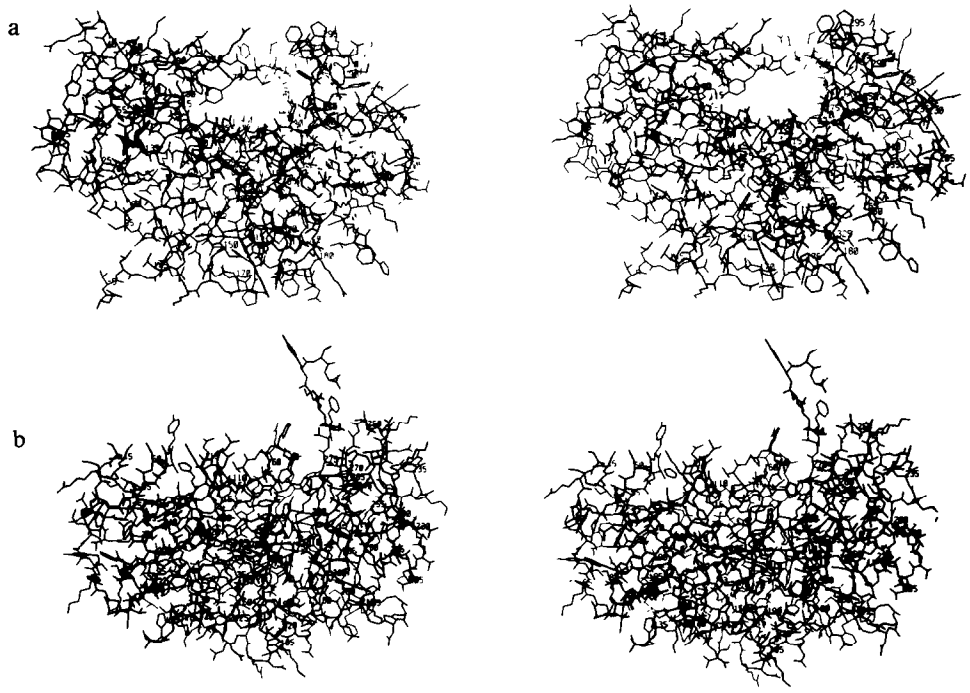


Fig.1. Stereo view of the 3-dimensional model of human renin viewed (a) along the active site cleft and (b) perpendicular to the cleft.

	10	1	2	2A	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Human renin		F	G	E	V	T	E	M	P	A	L	P	F	M	L	A	–	E	F	D
Human pepsin		F	G		L	S	E	T	E	P	G	S	F	L	Y	Y	A	P	F	D

This introduces an insertion and deletion, but conserves residue 111 as phenylalanine, a residue which contributes to the core and which is conserved in all the pepsins. Nevertheless the following alignment is equally plausible:

	10	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Human renin		F	G	E	V	T	E	M	P	A	L	P	F	M	L	A	E	F	D
Human pepsin		F	G	L	S	E	T	E	P	G	S	F	L	Y	Y	A	P	F	D

This presents some difficulties, particularly in the placement of polar and non-polar residues at equivalent positions. The ambiguity cannot be resolved at present and in any case the great differences in sequences in this region may indicate different conformations which must be defined by a detailed X-ray analysis of the human renin.

In general, the model building gives convincing evidence that there is a close 3-dimensional resemblance between renin and other aspartic proteinases. Most of the 92 residues which make substantial contributions towards the core of the aspartic proteinase fold are conserved as hydrophobic. Where large residues are replacing smaller ones there are compensatory changes in positions which are contiguous in the tertiary structure so that the core occupies the same volume. Most hydrophobic core residues which are conserved in all other aspartic proteinases are conserved in renins; these include Tyr 14, Phe 31, Trp

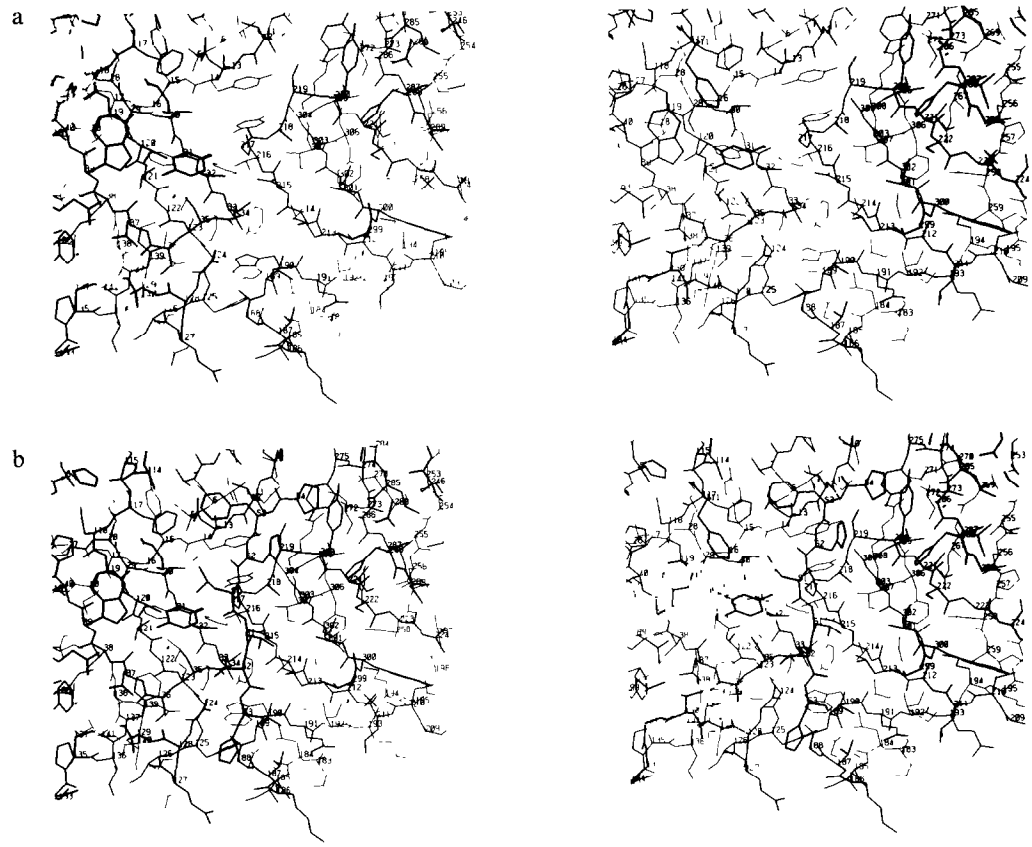


Fig.2. (a) The residues which comprise the inner surface of the active site cleft of human renin and (b) a similar view with an angiotensinogen sequence bound.

39, Val 40, Val 91, Phe 151, Trp 190, Tyr 275, Phe 306 and Phe 314. The exception, Val 89, is conservatively varied to isoleucine in renin. Several polar residues are hydrogen bonded into the core and conserved in all aspartic proteinases; these include serine 42 as well as Thr 33 and Thr 216 which are hydrogen bonded together in the active site region [29].

Asp 11 is conserved as an acid group in human renin as it is in all other aspartic proteinases with the exception of mouse submaxillary renin; this appears to form internal hydrogen bonds. Asp 87, Asp 118, Asp 171 and Asp 315 are also conserved in all aspartic proteinases for reasons which are probably structural. However, Asp 304 of pepsin and all other aspartic proteinases is alanine in human as well as mouse submaxillary renin. We have suggested that this is necessary for raising the pH optimum of mouse submaxillary renin to a more neutral pH compared to other aspartic proteinases as this residue is in a mainly hydrophobic environment and close to the active site. Its existence as alanine in the human renin supports this contention.

The conformation of the main chain of renin can be close to that of other aspartic proteinases in most parts. This is made possible by the conservation of glycines at positions 21, 34, 78, 82, 119, 122, 168, 217 and 322 in all aspartic proteinases. The high proportion of glycines amongst the invariant residues is expected as they uniquely have no side chain; they may adopt unusual torsion angles and pack in ways unavailable to other amino acid residues. It is of interest that Gly 76 is invariant in all other aspartic proteinases; the existence of Ser 76 in human renin is likely to change the conformation at this β-hairpin which is close to the active site (vide infra).

The cystine disulphide bridge, Cys 250–Cys 283, is conserved in renins as in all other aspartic proteinases. This plays a role in holding the residues in the sequence between the half-cystines into the 3-dimensional structure. The disulphide bridges Cys 45–Cys 50 and Cys 206–Cys 210 are accommodated with little change of conformation.

For modelling human renin endothiapepsin offers several advantages compared to other structures defined at high resolution. For example, the deletions at residues 10, 25, 26, 64, 207 and 208 of penicillopepsin are not found in endothiapepsin. An insertion of two extra residues in endothiapepsin in the β-bend between 278 and 283 compared to pepsin occurs at a position where residues are also inserted in human and mouse renins. At most positions where there are insertions in human renin when compared to endothiapepsin, the changes are unique to renins. These include two residues uniquely inserted in a surface loop (46a, 46b) and the insertions of residues at 159 and 279–282 which involve simple extensions of the β-hairpins. The existence of the proline-rich sequence PPPTGP (293–298), which prevents a pepsin-like conformation, is a further characteristic structural feature of renins.

In summary, human renin, like mouse submaxillary renin [6], may adopt a 3-dimensional structure closely similar to that of other aspartic proteinases. The differences are mainly confined to changes of side chains, which do not affect the main chain fold. Where insertions or deletions occur they are accommodated at the surface. We must now consider the implication of the model constructed using computer graphics for the biology of human renin.

In the active site of aspartic proteinases, Asp 32 and Asp 215 are hydrogen bonded together through their carboxyl groups which are coplanar and symmetrically arranged [22,29]: these are conserved in renins. The symmetrical arrangement is extended by the conservation of topologically equivalent threonines at 33 and 216 which hydrogen bond the two strands of polypeptide together, and glycines 34 and 217 which allow the chain to fold sharply so that 34 NH and 217 NH are close to the carboxylates Oδ1 of Asp 32 and Asp 215, respectively. All of these residues are conserved in both human and mouse renins. However, in previously sequenced aspartic proteinases, including mouse submaxillary renin, the arrangement is completed by serines or threonines at 35 and 218 which hydrogen bond through their side chain hydroxyl groups to the carboxylate Oδ2 of Asp 32 and Asp 215, respectively. In human renin residue 35 is serine, but residue 218 is uniquely alanine. This introduces a slight asymmetry and may contribute to a small change of the properties of the catalytic residues. It cannot, however, be responsible for the major differences of pH optimum between renin and other aspartic proteinases as this sequence difference is not shared by mouse submaxillary renin [4,26] or mouse kidney renin (C. Burt and W.J. Brammar, unpublished) [11].

Although the catalytically essential Asp 32 and Asp 215 and most of the amino acids close to them are identical in human and other aspartic proteinases, there are differences in the residues which line the active site cleft and define the substrate specificity. Table 2 lists the residues which define

Table 2

Possible specificity subsites in human renin

	Residues close to substrate (<4.5 Å)	Residues in proximity of substrate (>4.5 Å)
S_4	Tyr 220	Thr 284, Met 10, Asp 245, Ser 219, Tyr 275
S_3	Thr 12, Gln 13, Phe 111	Ser 219, Pro 110, Ala 114
S_2	Thr 77, Ser 76, Ala 218	His 288, Tyr 220
S_1	Tyr 75, Thr 77, Val 120, Val 30, Phe 111, Asp 32	Trp 39
S_1'	Leu 213, Asp 215	Ala 301, Ser 222, Thr 216, Thr 299, Val 189
S_2'	Ser 35, Leu 73, Gln 128, Arg 74	
S_3'	Thr 187, Val 189, Gln 191, Leu 213, Glu 127	

the subsites S_4–S_3'. The locations of these subsites are deduced from studies with other aspartic proteinases. Modelling of a substrate into endothiapepsin showed that the cleft accommodated 7 or 8 residues and pockets were identified for the subsites S_1 and S_1' on either side of the scissile bond [30,31].

The positions of the other subsites are in the cleft, but involve less well defined pockets. The assignments for S_4–S_1 were refined and largely confirmed by direct X-ray studies of inhibitors bound to penicillopepsin [28] and to rhizopuspepsin [27]. There are no direct studies which unequivocally define S_1'–S_3', but the original assignments have been improved by more precise modelling using the refined coordinates of endothiapepsin. It is interesting that the subsites are in similar positions in the NH_2- and COOH-terminal lobes of the aspartic proteinases [29,32,33].

In human renin subsite S_1 is a large hydrophobic pocket surrounded by Tyr 75, Thr 77, Val 120, Val 30 and Phe 111. This pocket is certainly not smaller than that in human pepsin as might be expected from the preference for leucine in angiotensin. Indeed, it is remarkably conserved with the exception of a valine at 120 instead of the leucine or isoleucine found in other aspartic proteinases.

Subsite S_1' is also large, involving Leu 213, Ala 301, Ser 222. The presence of leucine at 213, which replaces isoleucine in other aspartic proteinases and valine in mouse renin, is the only factor which could account for specificity for a smaller side chain at P_1' of different shape.

Most residues in the subsites S_2, S_3 and S_4 are identical or very similar in mouse and human renins although they differ from those of other mammalian aspartic proteinases [6]. Greater differences between renins and other aspartic proteinases are found on the COOH-terminal side of the scissile bond, in particular at S_3', where valine 189 replaces serine in mouse renin and a tyrosine or phenylalanine in other aspartic proteinases. The smaller hydrophobic residue allows the substrate residue at P_3' to be large and to attain a different position compared to other aspartic proteinases. The histidine in the human angiotensinogen sequence could easily be accommodated and the positive charge of the imidazole may be complemented by Glu 127 which is uniquely acidic in human renin.

There are many interesting differences between renins and other aspartic proteinases in the surface regions adjacent to the active site cleft. These may be important for two reasons. First, they may be involved in conformational changes on substrate binding and provide further interactions at P_4, P_2 and P_2' accounting for specificity at these positions. Secondly, they may participate in binding the macromolecular angiotensinogen molecule. In fact, the large size of angiotensinogen (453 residues) would imply extensive intermolecular surface contacts during proteolysis.

Fig.3 shows this surface region of human renin which comprises polypeptide loops characteristic of renins. PPPTGP is also found in mouse renins and must provide a rather rigid structure. The adjacent loop involves the sequence Lys 240, Lys 241,

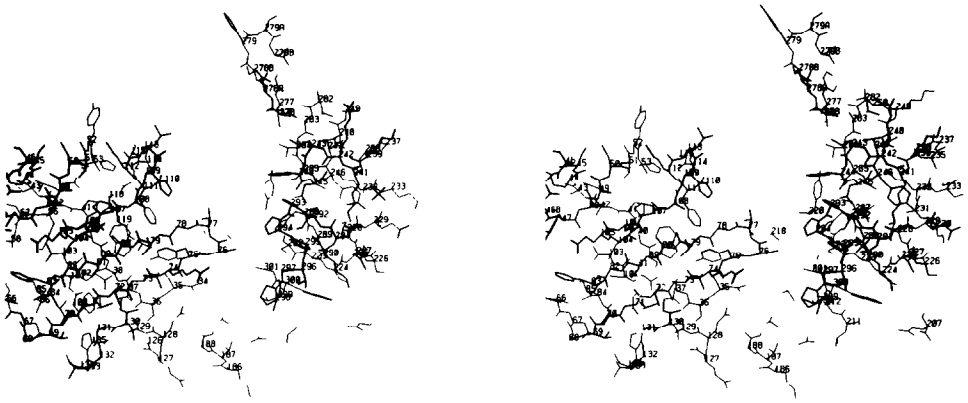


Fig.3. The arrangement of the residues at the edge of the active site viewed in the same direction as fig.2.

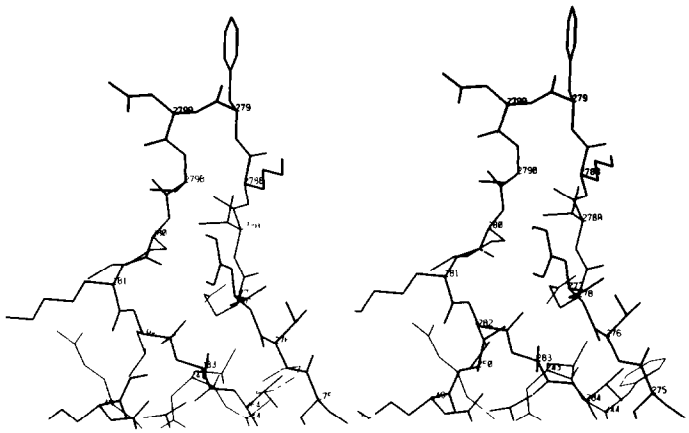


Fig.4. The β-hairpin in human renin which may be cleaved to give a 2-chain enzyme.

Fig.5. A stereo view of the model of human renin showing only α-carbon atoms and virtual bonds. The positions of intron-exon junctions are indicated by numbers on the two closest amino acid positions.

Arg 242 which is also a highly basic region in mouse renins. Other basic residues occur at Arg 74 and in the extended β-hairpin Glu (278)-Glu-Ser-Tyr-Ser-Ser-Lys-Lys-Leu (283). In mouse submaxillary renin, the chain is cleaved in this region to give two chains. In the mouse submaxillary enzyme this form has a lowered activity and changed specificity [34] which may result from rearrangement of the cleaved chain into the active site cleft. In the human enzyme the two lysines are less accessible and proteolytic cleavage may not occur so easily. Human renin has two presumptive *O*-glycosylation sites, Asn^{-1} Thr^{0} Thr^{1} and Asn^{67} Gly^{68} Thr^{69} [12]. These occur at surface regions which make them readily accessible to the glycosyl transferases of the endoplasmic reticulum.

Authors in [12] have sequenced the human renin gene and so it is possible to compare this with the available complementary DNA sequences for human renin. A three residue insertion - residues Asp 158, Ser 159a and Glu 159b in table 1 - either exists as a small exon yet to be located or is evidence of polymorphism. As these residues lie at a β-bend their presence has little effect on the integrity of the structure.

Comparison of the gene structure with the complementary DNA sequence confirms the existence of 8 introns in the gene for preprorenin, in positions closely similar to those found in the pepsinogen gene [16].

We may now consider the positions of the 7 introns which occur within the coding region for

renin with respect to the 3-dimensional model of human renin. In table 1 small arrows indicate the positions of intron-exon junctions, and their positions are shown in fig.5. The arrangement of the exons is not precisely related as would be expected by the gene duplication suggested in [32] although others [11] have suggested that the clustering of the exons supports this hypothesis. The junctions at 11–12, 90–91, 158–159, 196–197 and 278–278a are all on β-hairpins, i.e., close to the turns between two hydrogen-bonded antiparallel strands of β-sheet. They are all in surface regions accessible to the solvent. The intron-exon junctions at 50–51 and 245–246 are also on the surface strands of the renin molecule. Most of the amino acid sequences encoded by nucleotides flanking a splice-site mRNA are highly variable. The 3-residue length insertion noted in human renin occurs at the splice junction 159. The residues inserted in region 280 of renin relative to other aspartic proteinases also occur at a splice junction. Other intron-exon junctions are close to regions of high sequence variability, a fact which can be easily seen from table 1, where the conserved regions amongst aspartic proteinases are indicated by circles around the residues. Thus the members of the aspartic proteinase gene family such as pepsin and renin appear to be excellent examples of the sliding-junction model of gene family evolution proposed in [35] for other enzymes.

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